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ORIGINAL ARTICLE

Combined effect of longevity-associated mitochondrial DNA 5178 C/A polymorphism and coffee consumption on the risk of hyper-LDL cholesterolemia in middle-aged Japanese men

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The objective of this study was to investigate whether the mitochondrial DNA 5178 cytosine/adenine (Mt5178 C/A) polymorphism modifies the effects of coffee consumption on serum lipid levels and the risk of dyslipidemia in middle-aged Japanese men. A total of 397 male subjects (age, 53.9 ± 7.8 years; mean ± s.d.) were selected from among individuals visiting the hospital for regular medical check-ups. After adjustment for age, body mass index, habitual alcohol consumption, habitual smoking and use of antihypertensive medication, among subjects who consumed <1 cup of coffee per day, the odds ratio (OR) for hyper-low-density lipoprotein (LDL) cholesterolemia (serum LDL cholesterol ⩾140 mg per 100 ml) was significantly lower in those with Mt5178A than in those with Mt5178C (OR=0.378, 95% confidence interval: 0.153–0.919). After adjustment, the association between the Mt5178A genotype and hyper-LDL cholesterolemia depended on coffee consumption (*P* for trend=0.018). Coffee consumption was positively associated with serum LDL cholesterol levels only in subjects with Mt5178A. However, in subjects with Mt5178C, serum LDL cholesterol level or risk of hyper-LDL cholesterolemia did not seem to depend on coffee consumption. These results suggest that for men with Mt5178A, coffee consumption negates the genetic benefit of lower risk for hyper-LDL cholesterolemia.
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INTRODUCTION

Habitual coffee drinking is reported to be a preferable nutritional habit to decrease blood pressure,[1] and reduce the risk of type II diabetes[2] and metabolic syndrome[3] in the Japanese population. Moreover, a large-scale epidemiological study in Japan reported that coffee consumption is associated with a reduced risk of mortality from cardiovascular disease.[4] However, the effects of coffee consumption on the risk of cardiovascular diseases have not been confirmed clinically.[5] Considering the combined effects of coffee consumption and cytochrome P450 1A2 polymorphism on the risk of nonfatal myocardial infarction,[6] molecular epidemiological approaches seem to be essential for discussing the association between coffee consumption and coronary heart disease.

The mitochondrial DNA 5178 cytosine/adenine (Mt5178 C/A) polymorphism, also known as the NADH dehydrogenase subunit-2 237 leucine/methionine (ND2-237 Leu/Met) polymorphism, is associated with longevity in the Japanese population.[7–9] The frequency of the Mt5178A genotype is significantly higher in Japanese centenarians than in the general population.[7] Compared with those with Mt5178A, Japanese individuals with Mt5178C are more susceptible to lifestyle-related adult-onset diseases, such as hypertension,[10] diabetes,[11] myocardial infarction[12,13] and cerebrovascular disorders.[14] This polymorphism is reportedly associated with serum lipid levels[15] and fasting plasma glucose levels.[16] Moreover, the Mt5178 C/A polymorphism modulates the effects of alcohol consumption on blood pressure,[17] risk of hypertension,[10] serum triglyceride levels,[18] yearly changes in serum low-density lipoprotein (LDL) cholesterol levels[19] and serum uric acid levels.[20] Previous studies have also reported the combined effects of the Mt5178 C/A polymorphism and coffee consumption on the risk of hypertension[21] or abnormal glucose tolerance[22] in middle-aged Japanese men. For men with the Mt5178C genotype, ⩾2 cups of coffee may reduce the risk of

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hypertension.[21] Moreover, for such individuals, habitual drinking of ⩾4 cups of coffee may reduce the risk of abnormal glucose tolerance.[22] However, there have been no reports on the association between the Mt5178 C/A polymorphism and coffee consumption on serum lipid levels or the risk of dyslipidemia.

The objective of this study was to investigate whether there is a combined effect of longevity-associated Mt5178 C/A polymorphism and coffee consumption on serum lipid levels or the risk of dyslipidemia, a crucial risk factor for cardiovascular diseases, in middle-aged Japanese men.

SUBJECTS AND METHODS

Subjects

Participants were recruited from among individuals visiting the Mito Red Cross Hospital for regular medical check-ups between August 1999 and August 2000. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Kyorin University School of Medicine. Written informed consent was obtained from 602 volunteers before participation. Owing to the insufficient number of women recruited for classification into groups based on the Mt5178 C/A genotype and coffee consumption, women were excluded. Individuals taking antihyperlipidemic medication were also excluded, whereas those with diabetes were excluded because of their higher prevalence of dyslipidemia when compared with nondiabetic individuals.[23] A total of 406 men were enrolled in the study. Nine individuals with unclear data were subsequently excluded. Therefore, subjects comprised 397 Japanese men (age, 53.9 ± 7.8 years; mean ± s.d.).

Clinical characteristics of subjects

Determination of blood chemical and physical data proceeded as described previously.[15] LDL cholesterol levels were calculated by Friedewald's formula.[24] Dyslipidemias were defined according to the Japanese Atherosclerosis Society guidelines for prevention of atherosclerotic cardiovascular diseases (hyper-LDL cholesterolemia was defined as serum LDL cholesterol ⩾140 mg per 100 ml; hypo-high-density lipoprotein cholesterolemia was defined as serum high-density lipoprotein cholesterol <40 mg per 100 ml; and hypertriglyceridemia was defined as serum triglyceride ⩾150 mg per 100 ml).[25] Body mass index was defined as the ratio of subject weight (in kilogram) to the square of subject height (in meters). A survey of coffee consumption, alcohol consumption and habitual smoking was conducted using a questionnaire. Coffee consumption was classified on the basis of the number of cups of coffee per day: (1) <1 cup per day; (2) 1–3 cups per day; and (3) ⩾4 cups per day. Alcohol consumption was classified on the basis of drinking frequency (daily drinker; occasional drinker, which includes those who drink several times per week or per month; and nondrinker or ex-drinker). For habitual smoking, subjects were classified as nonsmoker or ex-smoker and current smoker, and for the use of antihypertensive medication, subjects were classified as taking no drug treatment or taking medicine.

Genotyping

Genotyping methods were used as described previously.[15] Briefly, DNA was extracted from white blood cells. PCR-restriction fragment length polymorphism using the restriction enzyme *Alu*I was performed. The absence and presence of an *Alu*I site was designated as Mt5178A and Mt5178C, respectively.

Statistical analyses

Statistical analyses were performed using SAS statistical software, version 9.1 for Windows (SAS Institute, Cary, NC, USA, 2002). Multiple logistic regression analysis was used to calculate the odds ratio (OR) for dyslipidemia. Differences in serum lipid levels between the coffee consumption groups were evaluated using the least-square means calculated from analysis of covariance. Differences with *P*-values of <0.05 were considered to be statistically significant.

RESULTS

No significant differences in biophysical or biochemical characteristics were observed between subjects with the Mt5178C and Mt5178A genotypes (Table 1).

The OR for hyper-LDL cholesterolemia was significantly lower in subjects with Mt5178A than with Mt5178C who consumed <1 cup per day (OR=0.393, 95% confidence interval (95% CI): 0.167–0.922; *P*=0.032) (Table 2). After adjusting for age, body mass index, habitual alcohol consumption, habitual smoking and use of antihypertensive medication, a significant OR remained (OR=0.378, 95%

Table 1 Clinical characteristics of study subjects by the Mt5178 C/A genotype

	Mt5178C N=*242*	*Mt5178A* N=*155*	P-*value*
Age (years)	54.4 ± 7.8	53.2 ± 7.8	0.160
Body mass index (kg m^{-2})	23.3 ± 2.8	23.5 ± 2.6	0.478
Systolic blood pressure (mm Hg)	125.9 ± 15.8	125.7 ± 14.1	0.926
Diastolic blood pressure (mm Hg)	73.9 ± 10.6	73.8 ± 9.1	0.878
Total cholesterol (mg per 100 ml)	203.6 ± 34.1	201.9 ± 31.8	0.654
LDL cholesterol (mg per 100 ml)	121.6 ± 34.6	117.9 ± 30.5	0.281
HDL cholesterol (mg per 100 ml)	54.6 ± 13.6	56.3 ± 16.2	0.275
Triglyceride (mg per 100 ml)	137.0 ± 90.9	139.5 ± 90.8	0.790
Fasting plasma glucose (mg per 100 ml)	97.7 ± 10.2	97.6 ± 9.7	0.961
Uric acid (mg per 100 ml)	5.98 ± 1.21	5.92 ± 1.21	0.658
Coffee consumption (<1 cup per day/1–3 cups per day/⩾4 cups per day) (%)	45.0/45.5/9.5	36.8/51.6/11.6	0.259
Alcohol consumption (daily/occasionally/nondrinker or ex-drinker) (%)	46.7/35.1/18.2	47.7/38.7/13.6	0.451
Current smokers (%)	41.3	40.7	0.894
Antihypertensive medication use (%)	19.4	12.9	0.091
Hyper-LDL cholesterolemia (%)	26.0	25.8	0.956
Hypo-HDL cholesterolemia (%)	11.6	12.3	0.836
Hypertriglyceridemia (%)	30.2	29.7	0.916

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein.
Age, body mass index, systolic blood pressure, diastolic blood pressure, serum total cholesterol levels, serum LDL cholesterol levels, serum HDL cholesterol levels, serum triglyceride levels, fasting plasma glucose levels and serum uric acid levels are given as means ± s.d. All *P*-values depict significance of differences between Mt5178C and Mt5178A. For coffee consumption, alcohol consumption, current smokers, antihypertensive medication use, hyper-LDL cholesterolemia, hypo-HDL cholesterolemia and hypertriglyceridemia, *P*-values were calculated by the χ^2-test.

Table 2 Odds ratios (ORs) and 95% confidence intervals (CIs) for hyper-LDL cholesterolemia by the Mt5178 C/A genotype and coffee consumption

	Frequency					
Genotype and coffee consumption	*Normal LDL cholesterol (LDL cholesterol <140 mg per 100 ml)*	*Hyper-LDL cholesterolemia* (LDL cholesterol ⩾140 *mg per 100 ml*)	*OR (95% CI)*		*Adjusted OR (95% CI)*[a]	
Mt5178C						
<1 cup per day (%)	77 (70.6)	32 (29.4)	1 (reference)		1 (reference)	
1–3 cups per day (%)	84 (76.4)	26 (23.6)	0.745 (0.408–1.361)		0.654 (0.345–1.239)	
⩾4 cups per day (%)	18 (78.3)	5 (21.7)	0.669 (0.229–1.955)		0.469 (0.137–1.609)	
			P for trend=0.298		*P* for trend=0.116	
Mt5178A						
<1 cup per day (%)	49 (86.0)	8 (14.0)	0.393 (0.167–0.922)*	1 (reference)	0.378 (0.153–0.919)*	1 (reference)
1–3 cups per day (%)	55 (68.8)	25 (31.3)	1.094 (0.584–2.048)	2.784 (1.150–6.741)*	1.009 (0.520–1.961)	2.872 (1.114–7.406)*
⩾4 cups per day (%)	11 (61.1)	7 (38.9)	1.531 (0.545–4.304)	3.989 (1.166–13.03)*	1.341 (0.420–4.280)	5.145 (1.221–21.68)*
				P for trend=0.012		*P* for trend=0.018

Abbreviation: LDL, low-density lipoprotein.
*$P<0.05$.
[a]OR adjusted for age, body mass index, habitual alcohol consumption, habitual smoking and antihypertensive medication use.

Table 3 Serum LDL cholesterol levels among three coffee-consumption groups by the Mt5178 C/A genotype

	Coffee consumption			
	<1 cup per day	*1–3 cups per day*	*⩾4 cups per day*	P *for trend*
Mt5178C	*N*=109	*N*=110	*N*=23	
LDL cholesterol	121.5±3.3	121.7±3.3	120.9±7.2	0.977
LDL cholesterol[a]	121.6±3.4	121.8±3.3	120.3±7.4	0.932
LDL cholesterol[b]	124.3±4.0	124.5±4.1	123.3±7.7	0.948
Mt5178A	*N*=57	*N*=80	*N*=18	
LDL cholesterol	111.3±4.0	121.3±3.4	123.7±7.1	0.050
LDL cholesterol[a]	110.9±4.0	121.2±3.3	125.5±7.1	0.030
LDL cholesterol[b]	110.6±5.4	120.3±4.6	124.1±7.8	0.049

Abbreviation: LDL, low-density lipoprotein.
[a]LDL cholesterol levels are given as least-square means±s.e. adjusted for age and body mass index.
[b]LDL cholesterol levels are given as least-square means±s.e. adjusted for age, body mass index, habitual alcohol consumption, habitual smoking and antihypertensive medication use. Bonferroni's correction for multiple comparisons was applied.

CI: 0.153–0.919; P=0.032). The OR for hyper-LDL cholesterolemia was significantly higher in subjects with Mt5178A who consumed 1–3 cups of coffee per day than in those who consumed <1 cup per day (OR=2.784, 95% CI: 1.150–6.741; P=0.023). After adjusting for age, body mass index, habitual alcohol consumption, habitual smoking and use of antihypertensive medication, a significant OR remained (OR=2.872, 95% CI: 1.114–7.406; P=0.029). Moreover, the OR for hyper-LDL cholesterolemia was significantly higher in subjects with Mt5178A who consumed ⩾4 cups of coffee per day than in those who consumed <1 cup of coffee per day (OR=3.898, 95% CI: 1.166–13.03; P=0.027). After adjusting for age, body mass index, habitual alcohol consumption, habitual smoking and use of antihypertensive medication, a significant OR also remained (OR=5.145, 95% CI: 1.221–21.68; P=0.026). The association between the Mt5178A genotype and hyper-LDL cholesterolemia may depend on coffee consumption (P for trend=0.012 and adjusted P for trend=0.018). On the other hand, the association between the Mt5178C genotype and hyper-LDL cholesterolemia does not seem to depend on coffee consumption (P for trend=0.298 and adjusted P for trend=0.116). No significant combined effect of the Mt5178 C/A polymorphism and coffee consumption on the risk of hypo-high-density lipoprotein cholesterolemia or hypertriglyceridemia was observed (data not shown).

Bonferroni's correction for multiple comparisons showed no statistically significant differences in serum LDL cholesterol levels among the three coffee consumption groups (<1 cup per day, 1–3 cups per day and ⩾4 cups per day) by the Mt5178 C/A genotype (Table 3). As the adjusted P-values for the trend reached a statistically significant level, a positive association between coffee consumption and serum LDL cholesterol levels was confirmed in subjects with the Mt5178A genotype, although no clear relationship between coffee consumption and serum LDL cholesterol levels was noted in those with the Mt5178C genotype.

DISCUSSION

This study showed that the Mt5178 C/A polymorphism and coffee consumption may combine to modify serum LDL cholesterol levels or the risk of hyper-LDL cholesterolemia in middle-aged Japanese men. Only among men who consumed <1 cup per day was the risk of hyper-LDL cholesterolemia significantly lower in those with Mt5178A than in those with Mt5178C. However, for men with the longevity-associated Mt5178A genotype, habitual consumption of ⩾1 cups of coffee may increase serum LDL cholesterol levels or the risk of hyper-LDL cholesterolemia. On the other hand, from the viewpoint of serum LDL cholesterol concentrations or the risk of hyper-LDL cholesterolemia, coffee consumption does not seem to adversely affect men with Mt5178C.

The observed frequency of Mt5178A did not significantly deviate from the values reported in other genetic epidemiological studies,[26] suggesting no genetic bias in the subjects in this study.

Compared with those with the Mt5178A genotype, individuals with the Mt5178C genotype are more susceptible to myocardial infarction.[12,13] However, for men with Mt5178C, coffee consumption apparently reduces the risk of hypertension[21] and abnormal glucose

tolerance,[22] and may therefore reduce the risk of life-threatening atherosclerotic diseases. In contrast, individuals with the Mt5178A genotype are genetically more resistant to atherosclerotic diseases than those with the Mt5178C genotype. The antiatherosclerotic advantages of the Mt5178A (ND2-237Met) genotype may be achieved on the basis of the biochemical properties of ND2-237Met, as methionine residues exert antioxidant potential for scavenging reactive oxygen species.[27]

Considering that hyper-LDL cholesterolemia is a major risk factor for atherosclerotic diseases,[25] abstaining from coffee consumption may be recommended to control serum LDL cholesterol levels in men with Mt5178A, although consuming <1 cup of coffee per day seems to have no effect on the genetic resistance to atherosclerotic disease. However, large-scale epidemiological studies have shown that drinking coffee contributes to the supply of antioxidant polyphenols in the Japanese population.[28] Moreover, coffee consumption promotes resistance to oxidative modification of LDL in humans.[29] Therefore, investigation of whether there is a combined effect of the Mt5178 C/A polymorphism and coffee consumption on LDL oxidation is required.

Meta-analysis of randomized controlled clinical trials has shown a dose–response relationship between coffee consumption and LDL cholesterol levels.[30] A large-scale cross-sectional study in Japanese men also showed that coffee consumption is associated with elevated levels of serum LDL cholesterol.[31] However, consideration of the genetic background seems to be necessary for interpreting the association between coffee consumption and serum LDL cholesterol. In this study, we report a significant and positive association between coffee consumption and serum LDL cholesterol levels only in men with Mt5178A. In addition to the Mt5178 C/A polymorphism, other genetic factors, for example, apolipoprotein A1 (APOA1) 83 cytosine/thymine (C/T) polymorphism or apolipoprotein E (APOE) polymorphism, should be considered. The LDL cholesterol-raising effects of coffee diterpene cafestol are significant in individuals with the APOA1 83 CC genotype compared with those with the APOA1 83 CT genotype,[32] whereas individuals with APOE *ε2* have slight resistance to the cholesterol-raising effects of coffee.[33] Therefore, gene–gene-diet or gene–gene–gene–diet interactions affecting serum LDL cholesterol levels should be investigated.

The biological mechanisms for the combined effects of Mt5178 C/A (ND2-237 Leu/Met) polymorphism and coffee consumption on serum LDL cholesterol levels remain unknown. They presumably depend on the biochemical differences in response to some compounds in coffee between ND2-237Leu and ND2-237Met. Indeed, elucidation of the mechanisms of the combined effects of Mt5178 C/A (ND2-237 Leu/Met) polymorphism and coffee consumption on the risk of hyper-LDL cholesterolemia remains a matter for further biochemical or pharmacological investigation.

In addition to the small sample size, a limitation of this study was the lack of information regarding the method of coffee preparation. Although filtered coffee consumption increases serum cholesterol,[34] individuals who drink boiled coffee show significantly greater elevation of LDL cholesterol levels than those who drink filtered coffee.[29] Whether the method of coffee preparation influences the combined effects of the Mt5178 C/A polymorphism and coffee consumption on serum LDL cholesterol levels or the risk of hyper-LDL cholesterolemia warrants further investigation. Another limitation was the lack of data on dietary patterns. In the Japanese population, coffee consumption is positively correlated with the meat diet pattern, which is characterized by higher consumption of processed meats, beef and pork, in comparison with the Western diet pattern, which is characterized by higher consumption of bread and butter; both diet patterns are associated with higher LDL cholesterol in Japanese men.[35] Consequently, details regarding dietary intake are required. In this study, LDL cholesterol was calculated using Friedewald's formula. Therefore, direct measurement for not only serum LDL cholesterol concentration but also oxidative LDL[36] is desirable.

In conclusion, we confirmed the combined effects of longevity-associated Mt5178 C/A polymorphism and coffee consumption on serum LDL cholesterol levels and the risk of hyper-LDL cholesterolemia. Among men who drink <1 cup of coffee per day, the risk of hyper-LDL cholesterolemia may be significantly lower in those with Mt5178A than in those with Mt5178C. However, for men with Mt5178A, coffee consumption may increase serum LDL cholesterol levels or the risk of hyper-LDL cholesterolemia. Although daily consumption of $\geqslant 2$ cups of coffee is recommended for men with Mt5178C to reduce the risk of hypertension[21] or abnormal glucose tolerance,[22] abstaining from coffee consumption may be recommended for those with Mt5178A to maximize the genetic predisposition toward lower levels of serum LDL cholesterol and the lower risk of hyper-LDL cholesterolemia. This mitochondrial DNA-based information may contribute to personalized prevention of life-threatening cardiovascular diseases, while promoting healthy aging and longevity.

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